UNIVERSITI PUTRA MALAYSIA

MASS CULTURE OF ARTHROSPIRA PLATENSIS UTILIZING AEROBICALLY DIGESTED PALM OIL MILL EFFLUENT (ADPOME)

KENNEDY AARON AGUOL

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By

KENNEDY AARON AGUOL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

March 2003

DEDICATION

Monongkotohuod zou kumaa KINOINGAN sabab nohdo balakat tosima kumaa doid dogo do nakaanu zou popotuhuk diti projek.

Au ku nogi hivan kumaa di koduvo-duvo zapa om zinaku
Bartholomew Quak@ Bartholomew Aguol om ***Addaline Mabel Chan***
Om nogi kumaa di tobinaiku
Grace Yvonne Aguol
Do minanak dogo doh sokodung om koginavaan ie togiot kopizo.

This thesis was written as a dedication to all researchers and scientist who had contributed directly and indirectly in the quest of knowledge.

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

MASS CULTURE OF *ARTHROSPIRA PLATENSIS* UTILIZING AEROBICALLY DIGESTED PALM OIL MILL EFFLUENT (ADPOME)

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Chairman : Dr. Hishamuddin Bin Omar

Faculty: Science and Environmental Studies

A series of experiments were conducted to develop a mass culture technique of *Arthrospira platensis*, a blue-green alga, in freshwater and brackishwater (15 ppt) using different concentrations (1, 2, 3, 4 and 5%) of aerobically digested palm oil mill effluent (ADPOME) in two trials. The cell growth, chlorophyll *a* , total protein, carbohydrate, lipid contents and fatty acid profile were also studied. Physio-chemical water quality parameters (temperature, dissolved oxygen, pH, salinity, surface irradiance, underwater irradiance, optical density, total ammonia nitrogen, nitrate nitrogen, nitrite nitrogen, total nitrogen, phosphate phosphorus, total phosphorus, chemical oxygen demand, biochemical oxygen demand and total dissolved organic carbon) were also measured throughout the study.

Algal cell growth was best in 4% ADPOME, both in the freshwater and brackishwater conditions and culture cycles with mean cell dry weight of 706.98 ±285.52 and 479.79±204.53 mg L^{-1}, respectively. The highest chlorophyll *a* content

was also observed in *A. platensis* cultivated in 4% ADPOME, in both freshwater and brackishwater conditions from both culture trials with mean chlorophyll *a* content at 10.12±6.10 and 8.40±5.23 mgL^{-1}, respectively.

A. platensis cultivated in 1% ADPOME yielded the highest total protein content in freshwater and brackishwater from both culture trials at 69.60±1.09 and 58.71±3.73 % dry weight ($p < 0.05$) when compared to other treatments. The highest total lipid content was observed in *A. platensis* that were cultivated in 5% ADPOME from both freshwater and brackishwater and culture trials at 7.03±0.13 and 10.08±0.01, respectively. Similarly, the maximum total carbohydrate content were also observed when *A. platensis* was cultivated in 5% ADPOME at 25.47±0.36 and 33.39±1.17% dry weight, respectively in both freshwater and brackishwater and culture trials. *A. platensis* cultured in 4% ADPOME had the most diverse fatty acid composition from both freshwater and brackishwater and culture trials.

Finally, the results of physio-chemical water quality from different concentrations of ADPOME both in the freshwater and brackishwater indicated that *A. platensis* has the ability to bioremediate organic rich palm oil mill effluent by reducing the retention time from 3-4 weeks to 1 week and improve the general water quality of the effluent.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGKULTURAN SECARA BESAR-BESARAN *ARTHROSPIRA PLATENSIS* MENGGUNAKAN KUMBAHAN MINYAK KELAPA SAWIT DIHADAM

Oleh

KENNEDY AARON AGUOL

Mac 2003

Pengerusi : Dr. Hishamuddin Bin Omar

Fakulti: Sains dan Pengajian Alam Sekitar

Satu siri eksperimen-eksperimen telah dijalankan untuk membangunkan kaedah pengkulturan secara besar besaran *Arthrospira platensis*, sejenis alga biru-hijau, dalam air tawar dan air payau (15 ppt) menggunakan kepekatan yang berbeza (1, 2, 3, 4 dan 5%) kumbahan minyak kelapa sawit dihadam aerobik (ADPOME) dalam dua percubaan pengkulturan. Tumbesaran sel, kandungan klorofil *a*, kandungan menyeluruh protein, lipid, karbohidrat dan profil asid lemak juga telah dikaji. Parameter-parameter mutu air fiziko-kimia (suhu, oksigen terlarut, pH, saliniti, irradian permukaan, irradian dalam air, ketumpatan optik, jumlah amonia nitrogen, nitrat nitrogen, nitrit nitrogen, jumlah nitrogen, fosfat fosforus, jumlah fosforus, keperluan oksigen kimia, keperluan oksigen biokimia dan jumlah karbon organik terlarut) turut disukat sepanjang kajian tersebut.

Tumbesaran sel terbaik telah diperolehi daripada 4% ADPOME, dalam kedua-dua persekitaran air tawar dan air payau dan kitaran pengkulturan dengan min berat sel kering pada 706.98±285.52 dan 479.79±204.53 mgL^{-1}, masing-masing.

Kandungan klorofil *a* tertinggi juga diperolehi daripada *A. platensis* yang dikultur dalam 4% ADPOME dalam kedua-dua persekitaran air tawar dan air payau dan percubaan pengkulturan dengan min kandungan klorofil *a* pada 10.12±6.10 dan 8.40±5.23 mgL^{-1}, masing-masing.

A. platensis yang dikultur menggunakan 1% ADPOME menghasilkan kandungan menyeluruh protein tertinggi dalam persekitaran air tawar dan air payau pada kedua-dua percubaan pengkulturan pada 69.60±1.09 dan 58.71±3.73 % berat kering ($p < 0.05$) apabila dibandingkan dengan rawatan lain. Kandungan tertinggi lipid menyeluruh diperolehi dalam *A. platensis* yang dikultur menggunakan 5% ADPOME dalam persekitaran air tawar dan air payau pada kedua-dua percubaan pengkulturan dengan min kandungan lipid 7.03±0.13 dan 10.08±0.01, masing-masing. Seperkara, kandungan menyeluruh karbohidrat maksima juga diperolehi dalam *A. platensis* yang dikultur menggunakan 5% ADPOME pada 25.47±0.36 dan 33.39±1.17 % berat kering, masing-masing. *A. platensis* yang dikultur menggunakan 4% ADPOME memiliki komposisi asid lemak yang paling pelbagai dalam kedua-dua percubaan pengkulturan dan persekitaran air tawar dan air payau.

Keputusan mutu air fiziko-kimia daripada kepekatan ADPOME yang berbeza dari kedua-dua persekitaran air tawar dan air payau menunjukkan *A. platensis* memiliki keupayaan dalam bioremediasi kumbahan minyak kelapa sawit yang kaya dengan bahan organik dengan mengurangkan masa simpanan daripada 3-4 minggu ke 1 minggu serta memperbaiki mutu air kumbahan secara umum.

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And above all, to ***GOD*** almighty for making all this possible.

I certify that an Examination Committee met on 3rd March 2003 to conduct the final examination of Kennedy Aaron Aguol on his Master of Science thesis entitled "Mass Culture Of *Arthrospira platensis* Utilising Aerobically Digested Palm Oil Mill Effluent (ADPOME)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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Date: 07 MAY 2003

The thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the supervisory committee are as follows:

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Date: 10 JUL 2003

DECLARATION FORM

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

KENNEDY AARON AGUOL

Date: 28th APRIL 2003

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ADPOME	Aerobically digested palm oil mill effluent
ASEAN	Association of South East Asian Nation
BF3	Boron trifluoride
BOD_5	Biochemical oxygen demand
CH_3	Methyl group
$CHCl_3$	Chloroform
COD	Chemical oxygen demand
COOH	Carboxyl
DHA	Docosahexaenoic acid
DV	United States Daily value
EFA	Essential fatty acid
EPA	Eicosapentanoic acid
FAMEs	Fatty acid methyl esters
FeEDTA	Ferric ethylenediaminetetra-acetic acid
FEP	Teflon
FFB	Fresh fruit bunches
GLs	galactolipids
GLA	Gamma linolenic acid
HCl	Hydrochloric acid
H_2SO_4	Sulphuric acid
IU	International unit
Ig E	Immunoglobulin E
mgL^{-1}	Milligram per litre
µm	Micro meter
µl	Micro litre
MUFA	Mono unsaturated fatty acid
N	Normality
NaOH	Sodium hydroxide
NED	N-1-Naphthylenediamine dihydrochloride
NO_2-N	Nitrite-nitrogen

NO_3-N	Nitrate-nitrogen
NPK	Nitrogen Phosphorus Potassium (Kalium)
PGE_1	Postaglandin
PO_4-P	Phosphate-phosphorus
POME	Palm oil mill effluent
ppt	Parts per thousand
PUFA	Poly unsaturated fatty acid
rpm	Revolution per minute
SFA	Saturated fatty acid
TAN-(NH_4-N)	Total Ammonia Nitrogen
T_dOC	Total dissolved organic carbon
TN	Total Nitrogen
TP	Total Phosphorus
UM	Universiti Malaya
UMS	Universiti Malaysia Sabah
UPM	Universiti Putra Malaysia
UNIDO	United Nation Industrial Development Organization
UV	Ultra violet
WHO	World Health Organisation
YSI	Yellow spring instrument